PROTOCOL

Protocol Number: P3659 GLP Study ID: GLP2960
VN2 18FEB2022

Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces

Test Microorganism(s)
Staphylococcus aureus ATCC 6538

Data Requirement
U.S. EPA OCSPP 810.2300

Study Sponsor
Allied Bioscience, Inc.
4460 Spring Valley Rd.
Farmers Branch, TX 75244

Testing Facility
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, Texas 78681

Author
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Date
11JAN2022
Revised Date: 12JAN2022

PROTOCOL (cont.)

I. Introduction

This document details the materials and procedure for evaluating the residual self-disinfection capability of a test substance using the US Environmental Protection Agency (EPA) Protocol 01-1A for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-porous Surfaces in accordance with Good Laboratory Practice Standards (GLPS) stipulated by 40 CFR 160. This document also explains the terms and conditions of testing.

II. Purpose

The purpose of this study is to document the residual self-disinfection efficacy of the test substance against the test system (microorganism), while utilizing a GardCo Washability and Wear Tester, under the test parameters specified in this protocol.

III. Justification for the Selection of the Test System (Microorganism)

The United States Environmental Protection Agency (US EPA) requires specific antimicrobial claims made for dried chemical residues sold in the United States to be supported by relevant test systems (microorganisms) as outlined in the United States Environmental Protection Agency Product Performance Test Guidelines, OCSPP 810.2300, Sanitizers for Use on Hard Surfaces – Efficacy Data Recommendations, and other related EPA guidance.

IV. Terms and Conditions

Studies by Microchem Laboratory are conducted in accordance with general terms and conditions posted on www.MicrochemLab.com/terms.

Prior to study initiation, Microchem Laboratory should receive the approved and signed protocol, test substances, and payment. Changes to the signed, approved protocol will require amendment and may incur additional fees. Cancellation of the study any time after the study initiation will result in a cancellation fee of up to 100% of the total study cost, to be determined by laboratory management at its sole discretion.

Microchem Laboratory may repeat studies at its cost in the event of an unintended protocol non-conformance that affects the study outcome, or for studies which yield invalid control results. If the Sponsor requests a specific neutralizer to be utilized in testing and test controls indicate incomplete or inadequate neutralization, repeat testing will be at the Study Sponsor's expense for applicable testing. Repeat testing may be conducted under the current initiated protocol and Microchem Laboratory GLP study identification number. In addition, the Study Sponsor is responsible for the cost of all studies performed to confirm the outcome of a previous study and for ensuring that the study will meet their regulatory objectives.

The Study Sponsor must obtain written consent from Microchem Laboratory to use or publish its protocols, study reports (or parts thereof), logo or employee names for marketing purposes.

PROTOCOL (cont.)

V. Test Substance Characterization and Handling

As stated in 40 CFR, Part 160, Subpart F [160.105] each batch (lot) of test substance shall be characterized with regards to identity, strength, purity, composition, and solubility (as applicable) and be documented prior to use in this this study. Stability of the test substance shall be determined prior to or concomitantly with this study. If the requirements set forth in 40 CFR Part 160 Subpart F [160.105] have not been met, this will be noted in the Good Laboratory Practices compliance statement in the study report. Certificates of Analysis (C of A) will be appended to the study report, if provided by the Study Sponsor.

Test substances are handled as follows unless otherwise specified by the Study Sponsor:

- The test substance is stored at ambient (room) temperature under fluorescent lighting or in a cabinet.
- The test substance is shaken or otherwise mixed well immediately prior to use, if applicable.
- The test substance is handled safely in accordance with the chemical risks it may pose, stated in the SDS or by the Study Sponsor during the course of pre-study communication.

VI. Study Dates

The proposed experimental start and termination dates are estimates based on the current laboratory schedule. These dates may change based on date the signed protocol, test substance(s), and applicable payment are received by the laboratory. To avoid scheduling delays, ensure that all paperwork is completed fully and accurately.

Proposed experimental start date: 21FEB2022
Proposed experimental termination date: 01MAR2022

VII. Procedure for the Identification of the Test System

Microchem Laboratory maintains Standard Operating Procedures which outline the procedures for receipt, storage, and tracking of microorganisms. The vessels, racks, and/or trays containing the test system are labeled with microorganism identifiers to maintain microorganism traceability. Information regarding the microorganism identity, strain, propagation procedure, media utilized, etc. is documented in the study raw data. Following testing, the microorganism identity of positive test replicates is confirmed following the appropriate macroscopic, microscopic, and biochemical assays. All studies are assigned a unique identification number which is labeled on the test and control vessels, racks, trays, etc. Additionally, Standard Operating Procedures are also in place for the receipt, storage, and usage tracking of all test and control substances utilized in testing. These procedures are followed to identify and document the test system.

VIII. Test System (Microorganism)

Microorganism	Growth Media	Incubation Conditions
Staphylococcus aureus ATCC 6538	Nutrient broth or Tryptic Soy Broth (Culture Media) Tryptic Soy Agar (Agar plating media)	Aerobic at 35 ± 2°C

The above microorganism(s) was received from the American Type Culture Collection (ATCC).

PROTOCOL (cont.)

IX. Procedure

Preparation of Test Surfaces

- The carrier type utilized in testing will be as requested by the Study Sponsor. Unless otherwise requested, individual approximate 1" x 1" mirrored stainless steel or non-frosted glass will be used in testing.
- The adhesive backing is removed from the stainless steel, if applicable.
- The carriers are cleaned by immersing in 70-95% ethyl alcohol (ethanol, reagent alcohol) or isopropyl alcohol.
- The carriers are thoroughly rinsed using tap water followed by two rinses in deionized (DI) water.
- The carriers are wiped dry using Kim wipes or other lint free cloth or wipe and visually screened for scratches, chips, or cracks. Flawed carriers are discarded.
- The carriers are autoclave sterilized for a minimum of 20 minutes at approximately 121°C.
- Each dry carrier is aseptically placed in a sterile Petri dish containing 2 pieces of sterile Whatman No. 2 filter paper, or equivalent.
- Alternatively, the following test surface preparation may be followed:
  - Following cleaning, the carriers may be decontaminated by immersing in absolute ethanol (95% ethanol) followed by sterile deionized water rinse. The carriers are allowed to air dry inside of a biosafety cabinet.
  - Each dry carrier is aseptically placed in a sterile Petri dish containing 2 pieces of sterile Whatman No.2 filter paper, or equivalent.

Preparation of Control Substance

- A 0.01% (v/v) Triton X-100 solution in deionized water is prepared and sterilized via filtration using a 0.22 µm filter on the day of treatment.
- A Preval spray bottle is sanitized with 95% ethanol or reagent alcohol, followed by a thorough rinse with sterile DI water to remove any excess ethanol.
- The prepared control solution is placed in the sanitized Preval spray bottle and used to treat the control carriers.

Preparation of Test Substance(s)

- The test substance will be used per Sponsor request.
- If a dilution of the test substance is requested by the Sponsor, the diluted test substance is used within three hours of preparation.
- Unless otherwise requested by the Sponsor, if a dilution of the test substance is required, a ≥1.0 ml or ≥1.0 g of the test substance is used for preparation using volumetric glassware. For liquid products, a v/v dilution is prepared and for solids, a w/v dilution is prepared.
- If synthetic hard water is requested as the diluent, it is prepared following Microchem Laboratory Standard Operating Procedures for the specific water type. The final hardness range is -10% to +5% of the specified hardness.
- If tap water is requested as the diluent, the water is sterilized prior to use. The water hardness is determined on the day of testing and adjusted to the hardness range if necessary. The hardness range is -10% to +5% of the specified hardness.

PROTOCOL (cont.)

Preparation of Daily and Test Cultures

For each microorganism

- A broth culture of the test microorganism is initiated by transferring an isolated colony from the monthly working stock plate to a tube containing a 10 ml volume of Nutrient broth or Tryptic Soy Broth. Transfer culture tubes are incubated at 35 ± 2 °C for 24 ± 2 hours. At least three consecutive daily transfers are conducted using a 10 µl loopful of microorganism into a 10 ml volume of Nutrient broth or Tryptic Soy Broth prior to the initiation of the test cultures.

Preparation of the Initial Inoculation Culture:

For each microorganism

- From the daily transfer series, a 48-54 hour culture is initiated at 35 ± 2 °C
- After the incubation period, the culture is vortex mixed for 3-4 seconds and allowed to stand for 15 ± 1 minutes at room temperature. The culture is diluted 1:10,000 by making two serial dilutions of 0.1 ml culture into 9.9 ml sterile DI water, vortex mixed, and allowed to stand at room temperature for a minimum of 15 minutes.
- If an organic soil load is requested by the Sponsor, it will be added to the organism prior to the second 15 minute holding time.

Carrier Inoculation with "Initial Inoculation Culture"

For each microorganism

- A 0.010 ml volume of the initial inoculation culture is applied to the test and control surfaces to within approximately 1/8 inch of the surface edge of each test and control carrier. A bent sterile micropipette tip is used to spread the inoculum.
- All inoculated carriers are dried uncovered at 35 ± 2°C for 30-35 minutes or until visibly dry.
- Only visibly dry carriers are used for the test.

Exposure of Test Carriers to Test Substance

For each microorganism

- Each lot of test substance is applied to four carriers, per Sponsor request, on a level surface.
- The solution on the test carriers is allowed to dry for a minimum of three hours, or until completely dry, at room temperature and 30-55% relative humidity with Petri dish lids ajar. The carriers may be dried overnight unless otherwise requested by the Sponsor.

PROTOCOL (cont.)

Exposure of Control Carriers to Control Substance

For each microorganism

- Four carriers are treated with sterile 0.01% Triton X-100 solution using the Preval spray bottle following the Sponsor's request.
  - If the test substance is applied to the test carriers via wipe application, the control carriers will be sprayed for approximately 3 seconds using a Preval sprayer at a distance of 6-8 inches and an angle of approximately 45°.
- The solution on the carriers from treatment is allowed to dry under the same conditions as the test carriers.

Preparation of the 24 Hour Re-inoculation Culture:

For each microorganism

- From the daily transfer series, an 18-24 hour culture is initiated.
- After the incubation period, the culture is vortex mixed for 3-4 seconds and allowed to stand for 15 ± 1 minutes at room temperature. The culture is diluted 1:10,000 by making two serial dilutions of 0.1 ml culture into 9.9 ml sterile DI water followed by a single 1:2 dilution of 5.0 ml of culture into 5.0 ml sterile DI water. If requested by the Sponsor, an organic load will be added to the culture. The culture is vortex mixed again for 3 – 4 seconds and allowed to stand for a minimum of 15 minutes at room temperature.
  - Fresh 18-24 hour cultures are prepared for the re-inoculation cultures to ensure no culture is allowed to stand with the organic soil load for longer than 8 hours.

PROTOCOL (cont.)

Abrasion and Re-inoculation

For each microorganism

- The GardCo abrasion tester is set to a speed of 2.25 to 2.5 for a total surface contact time of approximately 4-5 seconds, for one back and forth pass. Each abrasion/wear cycle in the test equals one pass to the left and a return pass to the right. With a calibrated timer, the contact time for one complete abrasion cycle is verified to be 4-5 seconds and the speed is adjusted as necessary. The pass contact time should be verified on each date of testing.
- Test carriers and control carriers undergo a wear and re-inoculation regimen. A series of 12 wear cycles and at least five re-inoculation cycles are completed prior to the efficacy test which is performed at least 24 hours after application of the test product unless otherwise requested by the Sponsor. This step is performed at room temperature and a relative humidity of 30-55%. A humidifier or other equipment may be utilized to maintain humidity.
  - See Tables 1 for specific wear and re-inoculation procedure.
- Temperature and relative humidity measurements are taken and recorded periodically throughout the abrasion process.
- The weight of the fully assembled abrasion boats are recorded and verified prior to initiation of the wear and re-inoculation regimen and must equal 1084 ± 0.2 g.
- All surfaces in contact with carriers on the GardCo apparatus are decontaminated with ethanol and allowed to dry completely between each set of surface wears to prevent carryover contamination.
- The foam liner and cotton cloths on the abrasion tester are replaced between each set of surface wears.
- After each complete set of abrasions are conducted (all control and test carriers abraded), the carriers are allowed to sit at least 15 minutes at ambient temperature prior to being re-inoculated.
- The carriers are re-inoculated with 0.010 ml of the re-inoculation culture and spread without allowing the inoculum to touch the edges of the carrier. Carriers are allowed to dry at room temperature for a minimum of 30 minutes or until completely dry prior to initiation of the next set of abrasions.
- Cotton cloths used as part of wet abrasions are prepared prior to each wet abrasion cycle by spraying the cloth with sterile DI water using a sanitized Preval sprayer, from a distance of 75 ± 1 cm for no more than 1 second, and used immediately.

PROTOCOL (cont.)

Table 1. Abrasion / Reinoculation Procedure for Residual Self Sanitization

This table outlines an example of the typical procedure used for reinoculation and wear cycles. Alternate schedules may be followed adhering to the protocol.

Day	Abrasion/Reinoculation Procedure
1	Inoculation of All Carriers with initial inoculation culture
	Test/Control Substance application and drying
2	Dry Abrasion (wear #1)
	Reinoculation (1)*
	Wet abrasion (wear #2)
	Reinoculation (2)*
	Dry Abrasion (wear #3)
	Reinoculation (3)*
	Wet abrasion (wear #4)
	Reinoculation (4)*
	Dry Abrasion (wear #5)
	Reinoculation (5)*
3	Wet Abrasion (wear #6)
	Dry Abrasion (wear #7)
	Wet Abrasion (wear #8)
	Dry Abrasion (wear #9)
	Wet Abrasion (wear #10)
	Dry Abrasion (wear #11)
	Wet Abrasion (wear #12)
	Sanitization Efficacy Test
*=with "reinoculation culture"	

PROTOCOL (cont.)

Preparation of the Final Test Culture:

For each microorganism

- From the daily transfer series, an 18-24 hour culture is initiated.
- After the incubation period, the culture is vortex mixed for 3-4 seconds and allowed to stand for 15 ± 1 minutes at room temperature. The upper two thirds of the culture are decanted or removed with a pipette into a new sterile tube and then supplemented with the Sponsor requested organic soil load, if applicable. The culture is vortex mixed again and allowed to stand for at least 15 minutes at room temperature.

Residual Efficacy Determination

For each microorganism

- Residual efficacy is determined for all carriers (test and control) after the last of the 12 wear and re-inoculation cycles, and at least 24 hours after the product application, corresponding to the study flow table above.
- Residual efficacy is determined by sequentially inoculating the carriers with 0.010 ml of the final test culture at an appropriate interval, spread without allowing the inoculum to touch the edges of the carrier, and then letting stand for the Sponsor requested contact time. The start and stop clock times for the contact time are recorded in the raw data.
- After the contact time has elapsed, carriers are aseptically transferred into jars containing a 30 ml volume of neutralizer broth using sterile forceps.
- Samples are sonicated for 20 ± 2 seconds in a sonicating waterbath. The samples are then agitated on an orbital shaker for 3 – 4 minutes at a speed sufficient for microbial recovery.
- Samples are serially dilutedby ten-fold dilution using 1.0 ml in 9.0 ml of sterile DI water. A 1.0 ± 0.1 ml aliquot of each appropriate dilution is then pour-plated in duplicate using standard dilution and plating techniques within 30 minutes of transfer to the neutralization broth.

Inoculum Concentration Determination

For each microorganism

- The concentration of the Initial Inoculation Culture, 24 Hour Re-Inoculation Culture(s), and Final Test Culture are determined by serially diluting 1.0 ml of sample in 9.0 ml volumes of sterile DI water and pour-plating 1.0 ± 0.1 ml aliquots of the appropriate dilutions in duplicate.

PROTOCOL (cont.)

X. Experimental Controls

Neutralization Controls

For each microorganism

- The effectiveness of the neutralizer is confirmed concurrently with or prior to efficacy testing.
- An 18-24 hour culture is initiated from the transfer series and used for the inoculation of the treated test and control surfaces.
  - Any 24 hr culture may be used for this control.
  - This control may be performed prior to or concomitantly with efficacy testing.
- For each test lot (batch) of test substance assayed, duplicate test surfaces are treated with the test product and duplicate control surfaces are treated with Triton X-100 solution.
  - If the test substance is applied as a wipe, the control carriers will be sprayed with the control Triton X-100 as in the test.
  - The surfaces are allowed to dry under ambient temperature.
- Treated and control test surfaces are aseptically transferred to 30 ml of neutralization broth, briefly mixed, and inoculated with an appropriate volume of dilute culture suspension containing approximately 1000-2000 organisms. Vessels containing carriers are briefly vortex mixed and allowed to dwell undisturbed for a hold time of 5±1 minutes.
  - This control may be performed using multiple treated test and control carriers inoculated with various organism dilutions.
  - Appropriate timed intervals may be followed for adequate and aseptic handling.
- After a hold time of 5 ± 1 minutes, 1.0 ml ± 0.1 ml aliquots of broth from each vessel are removed and pour-plated in duplicate to determine the number of microorganisms surviving in both the neutralization and inoculum controls.

Carrier Sterility Control(s)

For each microorganism

- A single untreated carrier is harvested in 30 ml of neutralization media and briefly vortexed. A 1.0 ml ± 0.1 ml aliquot is pour-plated using appropriate growth media and incubated to determine carrier sterility.

PROTOCOL (cont.)

Media Sterility Control(s)

- Media sterility controls are performed on each day of testing, as appropriate.
- A 0.1 ml volume of each lot of deionized water used is added to sterile growth agar and incubated alongside the test to confirm sterility of the serial dilution media at the time of testing.
- A 0.1 ml volume of each lot of culture diluent used is added to sterile growth agar and incubated alongside the test to confirm sterility of the culture diluent at the time of testing, if applicable.
- A 0.1 ml volume of each lot of test substance diluent used is added to sterile growth agar and incubated alongside the test to confirm sterility of the test substance diluent at the time of testing, if applicable.
- A 0.1 ml volume of the control substance prepared and used is added to sterile growth agar and incubated alongside the test to confirm sterility of the serial dilution media at the time of testing.
- A 0.1 ml volume of each lot of the organic soil load utilized in testing is added to sterile growth agar and incubated alongside the test to confirm soil sterility at the time of testing, if applicable.
- A 0.1 ml volume of each lot of subculture/neutralization broth used is added to sterile growth agar and incubated alongside the test to confirm neutralization broth sterility.
- A plate containing each lot of sterile growth agar used in this study is incubated alongside the test to confirm sterility at the time of test.

Media Viability and Culture Purity Control

- A loop full of each test microorganism culture prepared (Initial, re-inoculation, and final) is struck to the appropriate growth agar, on each day of testing, to achieve isolated colonies in order to confirm culture purity and media viability.

Incubation of Test Materials

- All plates are incubated for 48-54 hours at 35 ± 2 °C.
- Following incubation, the plates are visually enumerated and results are recorded in the raw data.
  - Test materials may be stored at 2 – 8°C for up to 3 days if results are not read immediately following incubation.
- A Gram stain or appropriate biochemical analysis may be performed for confirmation of the presence of the test microorganism at the discretion of the Study Director.

PROTOCOL (cont.)

XI. Calculations

If possible, use counts of 30 – 300 for calculations. Counts greater than 300 may be documented as >300 or as TNTC and are not included in calculations. Only countable dilutions are used for calculation purposes.

$$\text{CFU/ml for the culture suspension} = \frac{\text{(Average CFU/plate) x (dilution factor)}}{\text{volume plated in ml}}$$

CFU/Carrier = CFU/ml x 30 ml

Efficacy results are reported as the percent reduction of the geometric mean of the test microorganism on the test carriers calculated relative to the geometric mean of the test microorganism on the control carriers.

Percent Reduction is calculated as follows:

$$\text{Geometric Mean of Control Carriers} = \text{Antilog}\ \frac{(\log_{10}X_1 + \log_{10}X_2 + \log_{10}X_3 + \log_{10}X_4)}{4}$$

Where:

X= the Number of Microorganisms (CFU) Surviving Per Control Carrier

$$\text{Geometric Mean of Test Carriers} = \text{Antilog}\ \frac{(\log_{10}Y_1 + \log_{10}Y_2 + \log_{10}Y_3 + \log_{10}Y_4)}{4}$$

Where:

Y= the Number of Microorganisms (CFU) Surviving Per Test Carrier

$$\text{Percent Reduction} = \frac{(A-B)}{A} \times 100$$

Where:

A= Geometric Mean of the Number of Microorganisms surviving on the Control Carriers
B= Geometric Mean of the Number of Microorganisms surviving on the Test Carriers

Neutralization Control Calculations are as follows:

(A/B) x 100 = Percent comparison

Where:
A = average CFU per plate for Test Carriers
B = average CFU per plate for Control Carriers

XII. Proposed Statistical Analysis

Not applicable.

XIII. Methods for Control of Bias

Not applicable.

PROTOCOL (cont.)

XIV. Study Success Criteria

The experimental success (controls) criteria follow:

- The media and carrier sterility controls demonstrate no growth.
- The neutralizer sterility control demonstrates no growth.
- The soil sterility control demonstrate no growth.
- The media viability control must be positive for growth.
- The purity isolation streak for each culture purity control must demonstrate a pure culture as evidenced by colony morphology
- The recoveries from the Neutralization Control test treated carriers are ≥70% of the Neutralization control carriers
- A geometric mean concentration of at least 1×10^4 CFU/carrier must be recovered from the inoculated control carriers.

If any controls do not meet the specified experimental success criteria, testing may be repeated at the discretion of the Study Director under the same study protocol.

XV. Product Performance Criteria

The Environmental Protection Agency performance criteria for residual self-sanitization follow:

- The results should demonstrate a reduction of ≥99.9% when compared to the geometric mean of control carrier counts following a ≤5 minute contact time.

Retesting Guidance for Self-Sanitization:

- If the geometric mean concentration recovered from the inoculated control carriers treated with the control solution during the final residual sanitization is below 1×10^4 CFU/carrier, testing may be repeated at the discretion of the Study Director.

XVI. Reporting

Results are reported accurately and fully, in accordance with Environmental Protection Agency GLP (40 CFR Part 160). A draft report may be provided to the Study Sponsor for review prior to study completion.

PROTOCOL (cont.)

XVII. Study Record and Sample Retention

- The original (or certified copy) of the study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory indefinitely. For studies not meeting the performance criteria for submission or for studies that have been canceled prior to the generation of valid data, the original (or certified copy) of the final study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory for a minimum of two years following the study completion date at which time they may be removed from the archive or transferred to the Sponsors archive at their expense.
- If requested by the Study Sponsor (or Sponsor Representative), the study file may be transferred to the Study Sponsor's archive at the Study Sponsor's expense prior to the time frames listed.
- All test facility records including, but not limited to, standard operating procedures, quality assurance inspection records, temperature and equipment records including maintenance, inspection and calibration, and employee training records will be maintained at Microchem Laboratory indefinitely.
- The test substance (or test control, test article, test device, as applicable) may be returned to the Study Sponsor at the Study Sponsor's request and expense following study completion unless otherwise requested to be returned earlier. If the Study Sponsor does not request return of the sample, it will be disposed >90 days following the study completion. Arrangements may be made for extended storage as necessary, at the Sponsor's request and expense.

XVIII. Quality Assurance

The study is conducted in accordance with Microchem Laboratory's Quality Management System and EPA 40 CFR Part 160 and will undergo a full quality assurance review. All protocol amendments will be fully recorded and reported, as well as any deviations from the protocol.

XIX. References

- US EPA Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on hard, Non-Porous Surfaces. Protocol number 01-1A.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2000: General Considerations for Testing Public Health Pesticides – Guidance for Efficacy Testing. February 2018.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2300: Sanitizers for Use on Hard Surfaces – Efficacy Data Recommendations.
- U.S. Environmental Protection Agency, Frequent Questions for the 2018 series 810 – Product Performance Test Guidelines: Antimicrobial Efficiency Test Guidelines. 2019.
- Guidance Document – Disinfectant Drugs. Health Canada. April 2020.

PROTOCOL (cont.)

Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces

Protocol Number: P3659
Revised Date: 12JAN2022

Specific Testing Parameters to be completed by the Study Sponsor/Representative
- all fields need to be completed before testing may commence

Test Substance Name	Jaguar 5
Test Substance Batch Numbers	122108J5-LCL; 122109J5-LCL; 122110J5-LCL
Manufacture Date(s)	12/17/2021; 12/172021; 12/17/2021
Expiration Date(s)	N/A; N/A; N/A
Test Substance Shipment Status	X Use test substance already present at Microchem Laboratory. □ Test substance will be shipped. Estimated arrival date, if known:
Test Substance Storage	X Room temperature (default for all test substances unless otherwise requested) □ 2-8°C □ Other:
Test Substance Hazards	X None known □ SDS attached □ Other:
Test Substance Active Ingredient	□ Alcohol □ Iodophor □ Peracetic Acid □ Peroxide □ Phenol X Quaternary Ammonia □ Sodium Hypochlorite □ Other:
Active Ingredient Level	X At or below Lower Certified Limit (LCL) □ At or below nominal
Active Ingredient Concentration as submitted (for neutralization information only, not for chemical characterization)	n-Alkyl (50% C14, 40% C12, 10% C16) Dimethyl Benzyl Ammonium Chloride..... 0.144% Didecyl Dimethyl Ammonium Chloride..... 0.216%
Test Substance Dilution	X Ready to Use (RTU) □ Dilution ratio: (e.g. 1 oz per gallon)
Dilution to be made	X N/A □ Dilute by adding _____ test substance to _____ diluent = _____ total parts (please specify volumes to be used for dilution, e.g. 1 ml test substance to 127 ml diluent) Note, an equivalent dilution may be made unless otherwise noted.
Test Substance Diluent	□ 200 ppm sterile Tap Water (hardness range is 180-210 ppm) □ 400 ppm AOAC Synthetic Hard Water (hardness range is 360-420 ppm) □ 375 ppm OECD Hard Water (hardness range is 338 – 394 ppm) □ Other:

PROTOCOL (cont.)

Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces

Protocol Number: P3659
Revised Date: 12JAN2022

Continuation of Specific Testing Parameters to be completed by the Study Sponsor/Representative
- all fields need to be completed before testing may commence

Organic Soil Load	☐ None ☒ 5% fetal bovine serum ☐ Other:
Test Carrier Type	☐ Mirrored stainless steel ☒ Glass ☐ Other:
Product Application	☒ Spray: spray time or # of sprays: 2 • Spray Distance ☐ 4-6 inches ☐ 6-8 inches ☒ Other: 8 - 10 inches • Angle of Spray: approx. 60° ☐ Wipe: # of passes:
Contact Time	5 minutes *Note: Contact times of ≤ 1 minute include a ±3 seconds. Contact times of > 1 minute include a ±5 seconds.*
Neutralization/Subculture Broth	☐ Microchem to determine. Sponsor authorizes pre-test neutralization confirmation assay to be conducted to determine appropriate neutralizer, if needed. Additional fees may apply per price quotation. ☒ Use: D/E Neutralizing Broth
EPA 40 CFR Part 160.31(d) requires testing facility management to assure that the test, control, and reference substances have been appropriately tested for identity, strength, purity, stability and uniformity, as applicable.	Applicable identity, strength, purity, stability, and uniformity testing has been or will be completed prior to efficacy testing: ☒ Yes ☐ No - Performed under 40 CFR Part 160 Regulations? ☐ Yes ☒ No Stability testing of the formulation has been or will be completed prior to efficacy testing or concomitantly with efficacy testing: ☒ Yes ☐ No - Performed under 40 CFR Part 160 Regulations? ☐ Yes ☒ No If no is marked for either question, compliance status will be noted in the GLP compliance statement in the final report.
Certificate of Analysis (CoA)	☒ CoA for each batch provided. CoA will be appended in the final report. ☐ CoA will not be provided.
Protocol Modifications	☐ Testing to be performed as outlined in the protocol. ☒ The following protocol modifications are to be performed: Please see attachment: "Flairosol Sprayer Use Instructions_P3659" Please see attachment: "RSS Application Instructions_P3659"
Regulatory Agency(s) that report may be submitted to	☒ EPA ☐ Health Canada

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PROTOCOL (cont.)

XX. Authorized Personnel

Due to Microchem Laboratory confidentiality policy, study information will only be released to the Study Sponsor/Sponsor Representative who has signed the protocol unless otherwise noted in writing. Please list any additional personnel authorized to receive information regarding this study.

1. _____
2. _____
3. _____
4. _____

XXI. Protocol Approval

"I, the Study Sponsor, have read and understand the study protocol. By signing this protocol I am certifying that the information and parameters accurately describe the test(s) to be completed in accordance with Good Laboratory practice Standards (GLPS) stipulated by 40 CFR 160. I have also read, understand and agree to the terms and conditions listed in the protocol."

Study Sponsor/Sponsor Representative Signature Approving Protocol

Val Beck
Study Sponsor/Sponsor Representative Printed Name

Valeri Beck
Study Sponsor/Sponsor Representative Signature

02/07/2022
Date

vbeck@alliedbioscience.com
Email address

817-235-3375
Phone

Microchem Laboratory Study Director

L. Natalia Galván
Study Director Printed Name

Study Director Signature

18FEB2022
Date

PROTOCOL (cont.)

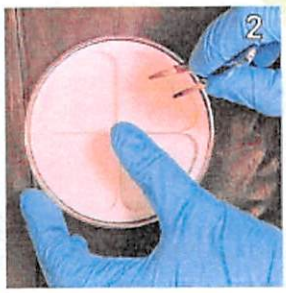
Allied BioScience

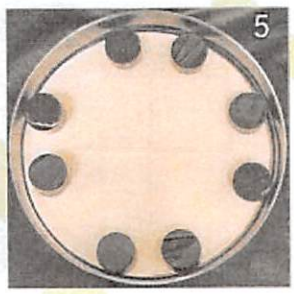
Protocol Attachment
Protocol ID: P3659
LNG 18FEB2022

RSS Application Instructions

Coupon arrangement:

- All four test carriers, per lot, are sprayed simultaneously. Sponsor provided a plastic guide that is to be used to arrange the coupons (Images 1-3, below). The guide improves the uniformity of application between lots of test substance.
  - The guide is place in the petri dish after initial carrier placement (Image 1, below). The guide can be sterilized by spraying with 70% Ethanol, but it should not be autoclaved.
  - Sterile forceps are used to adjust the position of the carriers in the petri dish (Image 2, below) Gentle pressure should be applied on the top of the handle while adjusting the position of carriers; without downward force, the carriers can slide under and touch adjacent carriers.
  - Two edges of each carrier should be in contact with the guide to ensure proper spacing (Image 3, below).
  - The guide is carefully removed before applying test substance (Image 4, below). It is critical that the carriers are not touching, there should be a 1-2 mm gap between each carrier.
  - After removal of the guide, place steel weights (sponsor provided) around edges of dish to secure and prevent rolling of the filter paper as test substance dries (Image 5, below). The weights should be added to the dish before test substance application; 8 weights are placed per dish. Steel weights are autoclavable and should be sterilized before use in testing.





PROTOCOL (cont.)

Allied BioScience

Protocol Attachment
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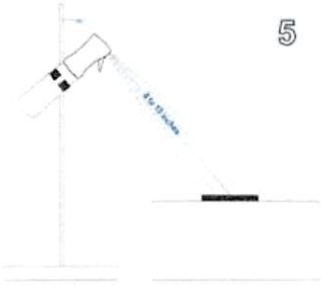
Applying test substance to carriers:

- Please follow "Flairosol Sprayer Use Instructions_P3659" to properly prime the sponsor provided sprayer prior to applying test substance to carriers.
- A clamp stand is used to hold the Flairosol Sprayer while applying test product to carriers.
  - The spray nozzle should be 8-10 inches from the center of the dish and the bottle should be at an angle of approximately 60 degrees (Image 5, below).
  - The most concentrated portion of the spray cone should be aimed at the center of the petri dish.
    - A test spray into an empty petri dish or bench top should be conducted before applying product to test carriers to confirm that the concentrated area of the spray cone lands in the center of the dish. If the volume is not concentrated in the center, the position of the dish should be adjusted (Image 6, below).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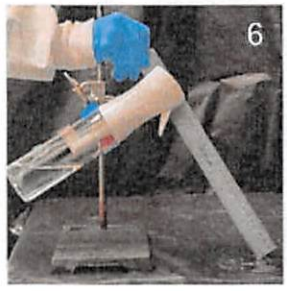
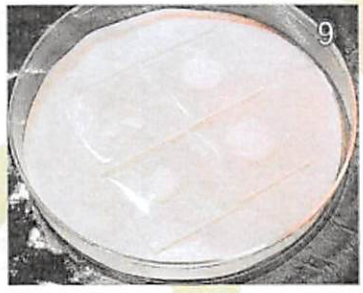

6


7

- Two sprays of test product are applied to each dish of four coupons (Image 7, above).
  - To ensure that the entire volume of test substance per spray is released from the sprayer, the applicator should fully depress the trigger and briefly pause (~1 second) between trigger pulls; failing to do so will result in reduced test substance volume. Two sprays from the sprayer should dispense approximately 2.0-2.5 mL of test substance; actual volume will vary slightly between triggers.
- Approximately 45 seconds to 1 minute after application is complete, any foam will settle to the center of the carrier (Image 8, below) and the test substance will appear as a bubble on top of the glass surface (Image 9, below).


8


9

- If test substance drains off the carriers or the foam does not settle as pictured above, test substance should be applied to a new set of carriers.

PROTOCOL (cont.)

Allied BioScience

Protocol Attachment
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- Carriers are left to dry uncovered, under ambient conditions overnight. The abrasion/reinoculation procedure is conduced the day after test substance application.
- After test substance application is complete, please follow the cleaning procedure in "Flairosol Sprayer Use Instructions_P3659" to properly clean the sponsor provided triggers prior to use in additional testing.

PROTOCOL (cont.)

Allied BioScience

Protocol Attachment
Protocol ID: P3659
LNG 18FEB2022

Flairosol Sprayer Use Instructions

Priming instructions:

- After test substance has been transferred to a Flairosol spray bottle, prime the Flairosol trigger:
  - Fully depress the trigger 3-4 times with a brief pause (~1 second) between each pull of the trigger to feed test substance into the straw.
  - Once test substance begins to dispense out of the nozzle, complete 20 additional sprays to ensure that all air has been removed from the system.
- This process should be repeated on each new day of testing.

Applying test substance to carriers:

- Flairosol spray bottles hold approximately 0.7 L of volume. For tests requiring a significant volume of test substance (i.e., GSPT), it is recommended that the bottles be filled with at least 0.5 L; this will ensure that there is enough liquid in the bottle such that the straw remains submerged in solution for the duration of testing.
- Flairosol spray bottles dispense test substance as a mist over test coupons. The spray cone provides coverage to the entire petri dish, with a concentration of product in the center of the dish. For residual sanitizer testing, please see "RSS Application Instructions_P3659" for additional guidance on coupon arrangement.
- To ensure that the entire volume of test substance per spray is released from the sprayer, the applicator should fully depress the trigger and briefly pause (~1 second) between trigger pulls; failing to do so will result in reduced test substance volume. Two sprays from the sprayer should dispense approximately 2.0-2.5 mL of test substance; actual volume will vary slightly between triggers.

Cleaning procedure:

- Flairosol triggers can be re-used, but should be cleaned between tests to maintain consistency.
- After testing is complete, remove trigger from bottle and depress the trigger 3-4 times to flush any remaining test substance from the straw.
- Place the trigger onto a different bottle (sponsor provided extra bottles) containing deionized water. Follow the priming instructions (see above) to clean the trigger.
- Again, remove trigger from the bottle and depress the trigger 3-4 times to flush any remaining water from the straw.
- The trigger is now clean and ready for use in another test.

CERTIFICATES OF ANALYSIS

Allied BioScience

CERTIFICATE OF ANALYSIS

Product Name: Jaguar 5

Lot Number: 122108J5-LCL

Date of Manufacture: 12/17/2021

Test	Specification	Result
Appearance	Colorless liquid	Colorless liquid
*Active Concentration (% w/w)	0.353 – 0.367	0.360
pH	4 - 6	4.7
Specific Gravity	0.9829 - 1.0230	0.9998

*Active ingredients: n-Alkyl (50% C14, 40% C12, 10% C16) Dimethyl Benzyl Ammonium Chloride, 40%; Didecyl dimethyl ammonium chloride, 60 %

The undersigned hereby certifies the following data to be true specification of the obtained results of the tests.

01/11/2022
Released Date for Shipment

Sumar Shrestha
Quality Control Analyst

CERTIFICATES OF ANALYSIS (cont.)

Allied BioScience

CERTIFICATE OF ANALYSIS

Product Name: Jaguar 5

Lot Number: 122109J5-LCL

Date of Manufacture: 12/17/2021

Test	Specification	Result
Appearance	Colorless liquid	Colorless liquid
*Active Concentration (% w/w)	0.353 – 0.367	0.363
pH	4 - 6	4.7
Specific Gravity	0.9829 - 1.0230	0.9999

*Active ingredients: n-Alkyl (50% C14, 40% C12, 10% C16) Dimethyl Benzyl Ammonium Chloride, 40%; Didecyl dimethyl ammonium chloride, 60 %

The undersigned hereby certifies the following data to be true specification of the obtained results of the tests.

01/11/2022
Released Date for Shipment

Sunan Shrestha
Quality Control Analyst

CERTIFICATES OF ANALYSIS (cont.)

Allied BioScience

CERTIFICATE OF ANALYSIS

Product Name: Jaguar 5

Lot Number: 122110J5-LCL

Date of Manufacture: 12/17/2021

Test	Specification	Result
Appearance	Colorless liquid	colorless liquid
*Active Concentration (% w/w)	0.353 – 0.367	0.360
pH	4 - 6	4.7
Specific Gravity	0.9829 - 1.0230	0.9998

*Active ingredients: n-Alkyl (50% C14, 40% C12, 10% C16) Dimethyl Benzyl Ammonium Chloride, 40%; Didecyl dimethyl ammonium chloride, 60 %

The undersigned hereby certifies the following data to be true specification of the obtained results of the tests.

01/11/2022
Released Date for Shipment

Suman Shrestha
Quality Control Analyst

REPORT AMENDMENT

The final report for GLP2960 was amended per the Study Sponsor's request to address findings outlined by the U.S. Environmental Protection Agency (U.S. EPA). These include the timeline for the wear regimen after the initial inoculation and application of test and control substance. The following changes and updates were made to the report:

- Report Changes section was included on Page 10 and reflected in Table of Contents
- In the procedure section on page 13, Figure 1 was updated to include the dates and start times for the initial inoculation, application of test and control substances, wear regimen (dry and wet abrasion), re-inoculations, and final sanitization test.

There is no change to the determination of efficacy, conclusion, or interpretation of results.

08JUN2022

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Company: Microchem Laboratory
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Date: